Reduced GLP-1 secretion at 30 minutes after a 75g oral glucose load is observed in gestational diabetes mellitus: a prospective cohort study

Running title: GLP-1 profile in gestational diabetes

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Abstract

Glucagon-like peptide 1 (GLP-1) levels may be reduced in type 2 diabetes but it has not been established whether a similar impairment exists in gestational diabetes mellitus (GDM). We studied this in a prospective cohort study of pregnant women (n=144) during oral glucose tolerance test (OGTT). GLP-1, glucose and insulin were sampled at 30-minute intervals during a 2-hour 75g OGTT and indices of insulin secretion and sensitivity calculated. In a nested case-control study, women with GDM (n=19) had 12% lower total GLP-1 secretion (area under the curve; AUC) compared to age, ethnicity and gestational-age matched controls (n=19), selected from within the lowest quartile of $glucose_{120min}$ values in our cohort. GDM had lower GLP-1 response in the first 30 minutes (19% lower $GLP-1_{30min}$ and 17% lower $AUC_{0to30min}$) after adjustment for possible confounders. Their glucose levels began to diverge at 30 minutes of the OGTT with increasing insulin levels, and by 120 minutes, their insulin levels were three times higher. In a secondary cohort of 57 women, which included 'high-normal' $glucose_{120min}$ values, low GLP-1 $AUC_{0to30min}$ was independently associated with lower indices of insulin secretion and sensitivity. In conclusion, we have observed that women with GDM have lower GLP-1 response at 30 minutes of an OGTT and hyperglycaemia at 120 minutes despite significant hyperinsulinaemia.

Gestational diabetes mellitus (GDM) is defined as diabetes first recognised during pregnancy, and is associated with alarmingly higher risk of type 2 diabetes and cardiovascular disease in the post-partum years (1). Around 5 – 30% of pregnancies globally are affected by GDM depending on the diagnostic criteria used (2; 3), but its pathogenesis has not been fully elucidated.

Glucagon-like peptide-1 (GLP-1) is one of two incretin hormones secreted by the L-cells of the intestines in response to food, particularly glucose and triacylglycerol (4; 5). Its primary function is to potentiate glucose-induced insulin secretion by pancreatic β-cells and together with glucose-independent insulinotropic polypeptide (GIP), accounts for around two-thirds of the insulin response after an oral glucose load (6).

The incretin effect, defined as the amplification of insulin secretion with oral compared to intravenous glucose, is reduced in type 2 diabetes, which may be due to decreased secretion of the incretin hormones or reduced responsiveness of the pancreatic β–cells to them (7; 8). Impairments in GLP-1 secretion may actually occur early in the disease process as shown in adults and adolescents with insulin resistance and obesity (9; 10). In addition, lower GIP levels and hyperglucagonemia have been demonstrated in type 2 diabetes, impaired glucose tolerance and obesity (11; 12).

The precise role of GLP-1 in pregnancies affected by GDM is still unclear. A search of the medical literature revealed five studies which measured GLP-1 levels in GDM (13-17). One showed a non-significant decrease in overall stimulated GLP-1 secretion between GDM and controls (13) and two did not show such a difference (14; 16).

Two measured only fasting GLP-1 (15; 17). All were retrospective studies, after a diagnosis of GDM was made and hence there is a need for prospective, adequately powered studies to validate the findings.

The primary aim of our study was to determine if there is a difference in GLP-1 response (as measured by total area under the curve, AUC_{total}) during a 2-hour oral glucose tolerance test (OGTT) between GDM and controls at the time of diagnosis. Secondary aims were to examine the time-course of GLP-1 during the OGTT and its relationship with indices of insulin secretion and sensitivity.

Research Design and Methods

Study design

A prospective cohort study, with a nested case-control component, was conducted on pregnant women between 2014 and 2016 in two hospitals in the West Midlands, UK. The National Institute for Health and Care Excellence (NICE) 2015 selective screening criteria was used to screen high-risk women for GDM at 26-28 weeks gestation (18). Exclusion criteria were pre-gestational diabetes mellitus (type 1 or type 2) and multiple gestation. Informed written consent was obtained from all participants. The study was approved by the National Research Ethics Committee (South Birmingham).

Blood sampling and laboratory analysis

On the day of OGTT, participants were studied after a minimum of 10 hours overnight fast. Plasma and serum samples were taken 0, 30, 60, 90 and 120 minutes of the OGTT. Analysis of serum glucose was done by a hexokinase enzymatic method

and insulin by enzyme linked immunosorbance assay (Abcam human insulin ELISA kit, Cambridge, UK). Plasma samples were stored at $-80^{\circ}C$ until the end of the study when they were transferred on dry ice to University of Copenhagen for analysis of GLP-1. GLP-1 measurements were done by radioimmunoassay as previously described (19).

Determination of GDM and control groups

Women were diagnosed with GDM according to the NICE 2015 criteria: fasting plasma glucose (glucose$_{0min}$) ≥5.6 mmol/l or 2-hour plasma glucose (glucose$_{120min}$) ≥7.8 mmol/l (18). The control group for the primary outcome (normal glucose tolerance, NGT group) was selected from those in the lowest quartile of glucose$_{120min}$ values in the cohort, matched for age, ethnicity and gestational age of OGTT to the GDM 'cases'. For the secondary outcome of assessing the relationship between GLP-1, insulin and glucose as continuous variables, the analyses were expanded to include additional participants who had the highest quartile of glucose$_{120min}$ values among the non-GDM (known hereafter as the NGT2 subgroup).

Statistical analysis

Based on a previous study (13), the primary effect size was determined to be 25% lower GLP-1 AUC$_{total}$ in women who develop GDM compared to controls. To detect this difference with 80% power at 5% significance (2-tailed), the estimated sample size was 20 cases. Since the detection rate of GDM in our cohort was around 15%, it was planned to recruit 150 women into the study.

AUC for GLP-1 was determined using the trapezoidal method and incremental AUC calculated as AUC above baseline. Surrogate markers of indices of insulin secretion and sensitivity were used in the absence of hyperglycaemic or hyperinsulinaemic-euglycaemic clamps, namely HOMA2-B (20) and HOMA-IR (insulin$_{0min}$ x glucose$_{0min}$ / 135). Insulin sensitivity was additionally measured using the Insulin Sensitivity Index (ISI) Stumvoll ($0.226 - 0.0032$xBMI $- 0.0000645$xinsulin$_{120min}$ $-$ 0.0037xglucose$_{90min}$) and oral glucose sensitivity index (OGIS) which uses glucose$_{0min}$, glucose$_{90min}$, glucose$_{120min}$, insulin$_{90min}$ and insulin$_{120min}$ values as well as participants height and weight (21-23). The ISI$_{Stumvoll}$ and OGIS correlate more highly with the gold-standard clamp studies (r=0.79 and r=0.70 respectively) than HOMA-IR, because they incorporate late phase glucose and insulin levels from the OGTT (21; 23; 24).

Statistical analysis was performed using SPSS version 22.0 (25). Comparison of GLP-1, glucose and insulin parameters between GDM and controls was done by analysis of covariance (ANCOVA) with post-hoc Bonferroni adjustment for multiple comparisons. All the analyses included the following co-variates: age, BMI, ethnicity, smoking and gestational week of OGTT.

Results

Characteristics of study population and OGTT results
One hundred and forty-four women completed the study, of whom 22 developed GDM. Three of these women were excluded from the primary analysis because their GLP-1$_{0min}$ or GLP-1$_{120min}$ values were unavailable (which are required for calculation of GLP-1 AUC$_{total}$), giving a total of 19 GDM cases and matched NGT controls

(Figure 1). An additional 17 participants (i.e. the NGT2 subgroup) were included for the secondary analyses. The maternal characteristics of the 38 women included in the primary analysis and 57 women included in the secondary analysis are presented in Table 1 and Supplementary Table 1 respectively.

GLP-1, glucose and insulin profiles: GDM and NGT

Women with GDM had 12% lower GLP-1 AUC_{total} (2034 vs 2321 pmol/l.120min; adjusted p=0.046) (Figure 2a) and 19% lower GLP-1 at 30mins (16.0 ± 3.90 vs 19.8 ± 4.52 pmol/l, adjusted p=0.042) (Figure 3a) compared to NGT. The early phase GLP-1 response, measured as $AUC_{0to30min,}$ was 17% lower in the former group (446 vs 536 pmol/l.30min; adjusted p=0.041) (Figure 2b).

The higher glucose levels of GDM were prominent from 30 minutes onwards of the OGTT but their insulin levels began to diverge at 90 minutes and peaked at 120 minutes (Figure 3b-c).

Relationship between GLP-1, glucose and insulin parameters: GDM, NGT and NGT2 subgroups

Secondary analyses to determine the associations between GLP-1, glucose and insulin were carried out by including the NGT2 group. This represents a group with intermediate glucose values at all time-points, although interestingly their insulin levels at 90 and 120 minutes were similar to GDM (Figure 3b-c). These women showed higher incremental GLP-1 rise overall and in the first 30 mins of OGTT compared to GDM group (incremental AUC_{total}: 398±455, 685±455, 427±433 pmol/l.120min; incremental $AUC_{0to30min}$: 34±46, 61±56 and 56±62 pmol/l.30min in

GDM, NGT2 and NGT respectively, p=0.04 GDM vs NGT2 for incremental $AUC_{total,}$

Figure 3a).

To investigate the relationship between GLP-1 and glucose and insulin, multiple

linear regression models were fitted looking at predictors of glucose and insulin at the

5 time-points in the secondary analysis of the cohort (n=57) (Supplementary Table 2).

None of the GLP-1 parameters were associated with glucose or insulin levels in the

first 60 minutes of the OGTT (data not shown). A temporal relationship between

lower GLP-1 $AUC_{0to30min}$ levels and hyperglycemia was observed. GLP-1 $AUC_{0to30min}$

was an independent negative predictor of glucose and insulin at 90 and 120 minutes in

separate models. However when glucose values were added to the insulin regression

model, the GLP-1 parameter lost significance.

Indices of insulin secretion and sensitivity

We next sought to investigate the influence of GLP-1 parameters on OGTT-derived

indices of insulin sensitivity and secretion in our cohort. Women with GDM had

lower insulin secretion as determined by HOMA2-B than NGT controls (p<0.001,

Table 1) as well as significantly lower $ISI_{Stumvoll}$ and OGIS values than NGT (p<0.001

for both) and lower $ISI_{Stumvoll}$ than the NGT2 subgroup (0.03 ±0.03 vs 0.06±0.04,

p=0.036). Combining all the patients (n=57) in multiple linear regression analyses,

GLP-1 $AUC_{0to30min}$ was an independent positive predictor of insulin secretion as

measured by HOMA2-B. Similarly, GLP-1$_{30min}$, GLP-1 $AUC_{0to30min}$ and GLP-1

AUC_{total} were also positively associated with OGIS, a marker of insulin sensitivity

and GLP-1$_{30min}$ with $ISI_{Stumvoll}$ (Supplementary Table 3).

Discussion

Our study reveals 3 key findings:- 1) Overall GLP-1 response is reduced in women with GDM compared to a control group selected from the lowest quartile of $glucose_{120min}$ values during an OGTT; 2) Impairment in GLP-1 secretion occurs in the first 30 minutes in GDM (as shown by lower $GLP-1_{30min}$ and $AUC_{0to30min}$ levels) and 3) The lower GLP-1 levels at 30 minutes may contribute to impaired glucose metabolism in pregnancy, regardless of GDM status.

GLP-1 profile in GDM pregnancy

Our primary outcome result of 12% lower GLP-1 AUC_{total} concentrations in GDM pregnancies was statistically significant after adjustment for possible confounders. Among other studies which reported AUC_{total} for GLP-1 response in GDM, Bonde et al (13) found a non-significant decrease of 25% in GDM but used a liquid meal test and sampled GLP-1 over 4 hours. Two other studies found no difference in the total response of GLP-1 in GDM during a 3-hour 100g and 2-hour 75g OGTT respectively (14; 16). The study by Cypryk et al had similar sampling times and GLP-1 assay as our study but possible reasons for the variance in their results could be a smaller sample size (n=13), and differences in baseline BMI, which was higher in our study (14).

Impact of low early phase GLP-1 response

A novel finding of our study was the lower GLP-1 response in the first 30 minutes of the OGTT in GDM pregnancies. Lower GLP-1 and insulin responses at 30 minutes of an OGTT has been shown in women with a history of GDM despite normal glucose values at 5 years post-partum (26). The authors suggest that this may put them at

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higher risk of progression to type 2 diabetes. However, this was not replicated in another cohort (27). Additionally, GLP-1 impairment in the first 30 minutes has been demonstrated in non-pregnant adults with pre-diabetes and type 2 diabetes where it was shown to influence β-cell function (9).

We observed a correlation between lower GLP-1 levels at 30 minutes and late phase high insulin levels during the OGTT. This negative relationship may seem paradoxical since one of the primary functions of GLP-1 is to stimulate insulin secretion by pancreatic β-cells. Although early phase GLP-1 levels were predictive of late phase hyperinsulinemia in our regression analyses, when glucose values (90 and 120 minutes) were introduced in the model, this relationship weakened. This suggests that the late phase hyperinsulinaemia is driven by hyperglycaemia. Therefore, our hypothesis is that the early phase GLP-1 response is critical to ensure that pancreatic β-cells produce appropriate amount of insulin to deal with a glucose load in a timely manner.

Although BMI has been shown to be negatively associated with GLP-1 in T2D (19), there was no correlation between 1st trimester BMI and any of the GLP-1 parameters in our cohort, nor was it a significant covariate in the ANCOVA analyses of mean GLP-1$_{30min}$, AUC$_{total}$, AUC$_{0to30min}$ between the three subgroups (data not shown). This lack of association is likely because women in our selectively screened, high-risk cohort were predominantly obese and therefore the effect of BMI on GLP-1 secretion may not be apparent.

Strengths and weaknesses

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This is the largest study, to our knowledge, investigating GLP-1 levels in GDM pregnancy using the widely available 75g OGTT. Its significant advantage is that at the time of GLP-1 sampling, neither the pregnant women nor the research team knew her GDM status, thereby minimising selection bias.

However, there are some important limitations which cannot be ignored. Our findings cannot be extrapolated to a diverse population of women with GDM, such as women from ethnicities other than White Caucasian, or those with predominantly fasting hyperglycaemia (15/19 women in our cohort had isolated post-prandial hyperglycemia, likely due to the NICE diagnostic criteria). Secondly, we have not analysed GIP and glucagon from this cohort and hence cannot be certain that impairments in the related incretin hormones will not contribute to our observations. However, two previous studies which have measured GIP during GDM (albeit with smaller number of patients) have not noticed any difference in GDM (13; 14). Additionally, it has been demonstrated that the insulinotropic effect of GIP, rather than its secretion, is impaired in type 2 diabetes (28). Therefore, we do not believe that plasma GIP results from OGTT will alter the conclusions. Fourthly, whilst our study demonstrated that GLP-1 secretion is lower in the early part of the OGTT in women with GDM, we cannot be sure that the trough does not occur before 30 minutes due to lack of sampling at earlier time points.

In summary, we have shown that GDM is associated with a lower GLP-1 response in the first 30 minutes of an OGTT, which may independently contribute to late phase hyperglycaemia, hyperinsulinaemia as well as reduced insulin sensitivity.

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Diabetes

Table 1 - Participant characteristics at baseline and during OGTT

Variables	GDM	NGT	p-value
Number	19	19	
Gestation at baseline (weeks)	12^{+5} (6^{+4}, 15^{+5})	12^{+3} (7^{+0}, 16^{+3})	NS
Age (yrs)	29.7 ± 5.3	28.4 ± 4.7	NS
Baseline weight	99.5 ± 17.9	81.2 ± 16.6	0.003
Baseline BMI (kg/m^2)	37.3 ± 7.5	29.1 ± 5.3	0.001
Waist circumference (cm)	114.7 ± 14.9	96.0 ± 12.9	<0.001
Current smokers (%)	4 (21.1)	1 (5.9)	NS
Ethnicity (%)			
European	17 (89.5)	14 (82.4)	NS
South Asian	2 (10.5)	2 (11.8)	
Afro-Caribbean	0	1 (5.9)	
History of GDM in a previous pregnancy	4 (21)	3 (16)	NS
Family history of diabetes mellitus	8 (42)	10 (53)	NS
Gestation of OGTT (weeks)	$26^{\pm6}$ (15^{+6}, 30^{+2})	$26^{\pm6}$ (15^{+6}, 29^{+5})	NS
Fasting glucose (mmol/l)	5.6 ± 1.03, 5.3 (4.5, 8.5)	4.7 ± 0.27, 4.7 (4.2, 5.1)	0.002
2 hour glucose (mmol/l)	9.3 ± 1.79, 9.0 (5.4, 12.7)	4.8 ± 0.34, 4.8 (4.1, 5.5)	<0.001
Fasting insulin (pmol/l)	50.0 ± 23.5	67.2 ± 51.2	NS
2 hour insulin (pmol/l)	490.0 ± 235.3	150.7 ± 124.1	<0.001
Insulin secretion			
HOMA2-B	72.3 ± 19.4	144.7 ± 53.5	<0.001
Insulin sensitivity (fasting values)			
HOMA-IR	2.18 ± 1.40	2.33 ± 1.77	NS
Insulin sensitivity (OGTT values)			
Insulin Sensitivity Index (ISI$_{Stumvoll}$)	0.03 ± 0.03	$0.10 \pm .019$	<0.001
Oral glucose insulin sensitivity (OGIS)	349.0 ± 81.7	472.3 ± 58.3	<0.001

Table showing characteristics of women with gestational diabetes mellitus (GDM, cases) and normal glucose tolerance (NGT, controls) at baseline and during oral glucose tolerance test (OGTT). Continuous variables are mean ($\pm$SD) or median (range) and categorical variables are n (%). T-test was used to compare the continuous variables and chi-squared test for categorical variables. .
NS: non-significant

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Figure legends

Figure 1 Flow diagram illustrating the selection of gestational diabetes mellitus (GDM) cases and normal glucose tolerance (NGT) controls for the primary analysis (n=38) and additional subjects for the secondary analyses of the study (n=57). The NGT control group includes women selected from the lowest $glucose_{120min}$ quartile, matched in age, ethnicity and BMI to the GDM cases. The NGT2 subgroup includes additional women who had $glucose_{120min}$ in the highest quartile but below the threshold for GDM diagnosis.

Figure 2 Univariate scatter plots of two parameters of GLP-1 response during an oral glucose tolerance test (OGTT) in pregnant women diagnosed with gestational diabetes mellitus (GDM) and controls with normal glucose tolerance, selected from the lowest quartile of $glucose_{120min}$ values (NGT). The parameters are (a) total area under the curve (AUC_{total}, mean 2034 vs 2321 pmol/l.120min; adjusted p=0.046) and (b) area under the curve in the first 30 minutes of the OGTT ($AUC_{0to30min}$, mean 446 vs 536 pmol/l.30min; adjusted p=0.041).

Figure 3 Line charts of (a) GLP-1, (b) glucose and (c) insulin concentrations during oral glucose tolerance test (OGTT) in women diagnosed with gestational diabetes (GDM, black circles), controls with normal glucose tolerance, selected from the lowest quartile of $glucose_{120min}$ values (NGT, white squares) and controls with normal glucose tolerance selected from the highest quartile of $glucose_{120min}$ values (NGT2, striped triangles) . The points represent the mean (±SEM) concentrations of the respective parameters measured at 30-minute intervals during a 2-hour 75g OGTT. Analysis of covariance was used to compare the means of each parameter between the GDM and NGT groups after adjustment for age, BMI, ethnicity and smoking.
* adjusted p<0.05
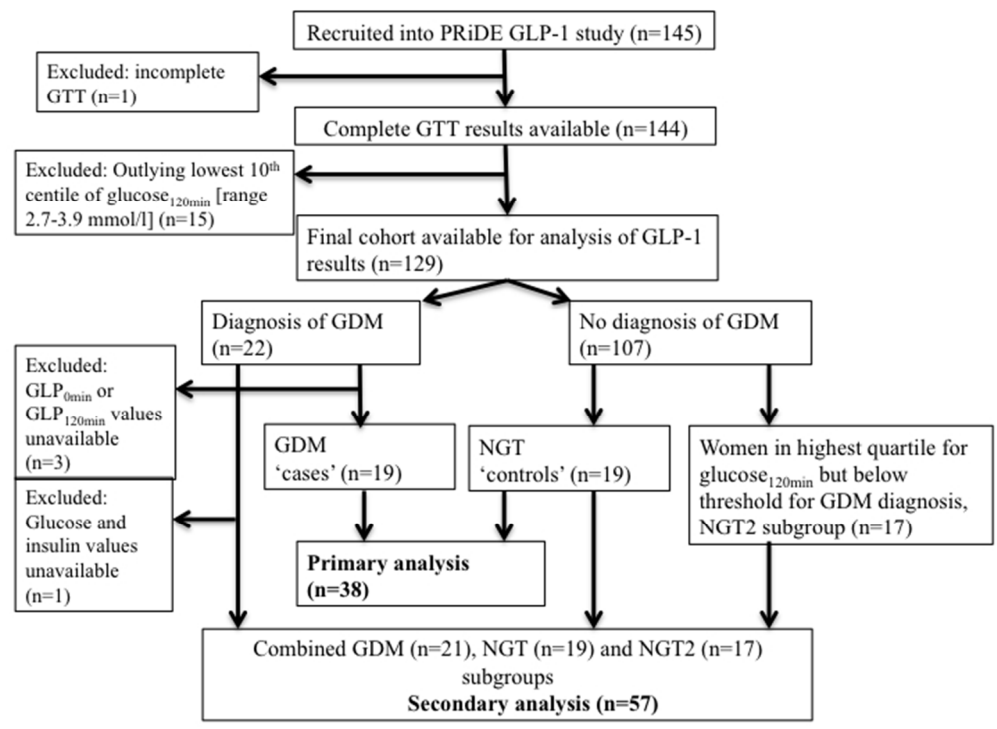** adjusted p<0.01
*** adjusted p<0.001

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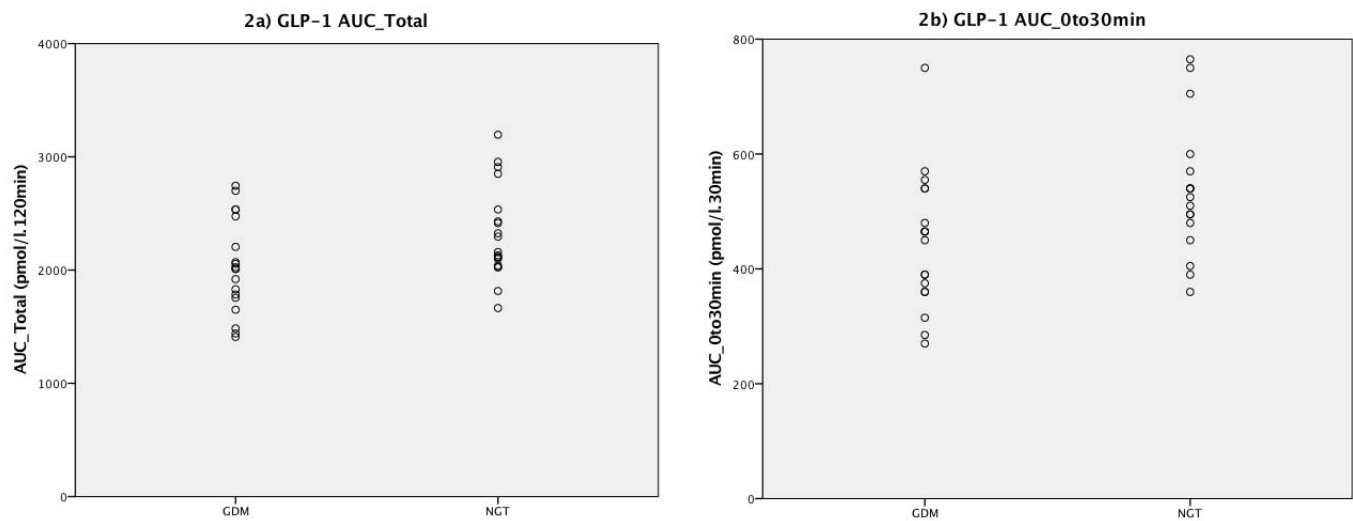
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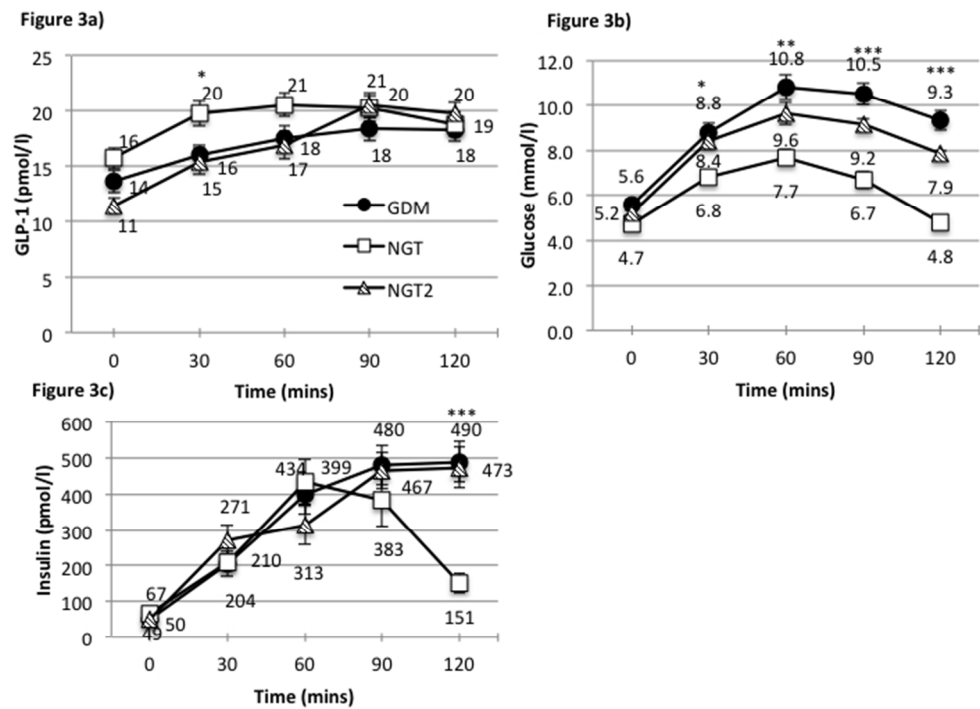
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* adjusted $p<0.05$
** adjusted $p<0.01$
*** adjusted $p<0.001$

254x191mm (72 x 72 DPI)

Supplemental Table 1 – Baseline characteristics, glucose and insulin results of participants included in the secondary analysis (n=57)

Variables	GDM	NGT	NGT2
Number	21	19	17
Age (yrs)	29.4 ± 5.2	28.4 ± 4.7	31.9 ± 4.3
Baseline BMI (kg/m^2)	38.2 ± 8.0	29.1 ± 5.3	32.7 ± 7.8
Waist circumference (cm)	116.3 ± 16.2	96.0 ± 12.9	107.1 ± 16.1
Current smokers (%)	4 (19.0)	1 (5.9)	0 (0)
Ethnicity (%)			
European	18 (85.7)	14 (82.4)	12 (80.0)
South Asian	3 (14.3)	2 (11.8)	1 (6.7)
Afro-Caribbean	0 (0)	1 (5.9)	1 (6.7)
Other	0 (0)	0 (0)	1 (6.7)
Gestation of OGTT (weeks)	$26^{+6} (15^{+6}, 30^{+2})$	$26^{\pm6} (15^{+6}, 29^{+5})$	$27^{+0} (24^{+0}, 33^{+0})$

Table showing the characteristics of the participants included in the secondary analysis, which examined the association between GLP-1, glucose and insulin levels as continuous variables. The participants' data are grouped according to their glucose status in the table but the analyses were done on the cohort as a whole.

GDM: gestational diabetes mellitus; NGT: normal glucose tolerance (the women who were age-, ethnicity- and gestational-age matched controls for the GDM cases in primary analysis, selected from the lowest quartile of glucose values); NGT2: normal glucose tolerance 2 (the women in highest quartile for $glucose_{120min}$ but below threshold for GDM diagnosis); OGTT: oral glucose tolerance test

Diabetes

Supplementary Table 2 – Multiple linear regression analyses of the predictors of serum glucose and insulin levels during OGTT

	Predictors of glucose$_{90min}$	Predictors of glucose$_{120min}$	Predictors of insulin$_{90min}$	Predictors of insulin$_{120min}$
Model A	GLP-1 AUC$_{0to30min}$ (β: -0.24, p=0.039)	GLP-1 AUC$_{0to30min}$ (β: -0.29, p=0.025)	GLP-1 AUC$_{0to30min}$ (β: -0.32, p=0.031)	GLP-1 AUC$_{0to30min}$ (β: -0.30, p=0.026)
Model B	N/A	N/A	GLP-1 AUC$_{0to30min}$ (β: -0.18, p=0.13)	GLP-1 AUC$_{0to30min}$ (β: -0.09, p=0.40)

Table showing the association between GLP-1 AUC$_{0oto30min}$ and glucose and insulin at 90 and 120 minutes during oral glucose tolerance test (OGTT) in the secondary cohort (n=57). The strength of association of GLP-1 AUC$_{0oto30min}$ the in the different models is given as the β-coefficient with the corresponding p-value.

Model A: The independent variable is GLP-1 AUC$_{0oto30min}$ and covariates are age, BMI, ethnicity, smoking status, gestation and glucose$_{0min}$ (for the insulin regression analyses insulin$_{0min}$ is a co-variate instead of glucose$_{0min}$)
Model B: As for Model A plus all glucose levels up to the corresponding time-point
N/A: not applicable

Supplementary Table 3 - Multiple linear regression analyses of relationship between indices of insulin secretion or sensitivity and GLP parameters

Variables	Unadjusted model		Adjusted model	
	Pearson's r	p-value	β-coefficient	p-value
Insulin secretion indices[a]				
HOMA2-B				
GLP-1$_{30min}$[b]	-	NS	-	NS
GLP-1 AUC$_{0to30min}$	0.33	0.026	0.38	0.004
GLP-1 AUC$_{total}$	-	NS	-	NS
Insulin sensitivity indices[a]				
HOMA-IR				
GLP-1$_{30min}$[b]	-	NS	-	NS
GLP-1 AUC$_{0to30min}$	-	NS	-	NS
GLP-1 AUC$_{total}$	-	NS	-	NS
ISI Stumvoll[c]				
GLP-1$_{30min}$[b]	0.33	0.02	0.31	0.035
GLP-1 AUC$_{0to30min}$	-	NS	-	NS
GLP-1 AUC$_{total}$	-	NS	-	NS
OGIS[c]				
GLP-1$_{30min}$[b]	0.41	0.004	0.41	0.004
GLP-1 AUC$_{0to30min}$	0.35	0.016	0.34	0.021
GLP-1 AUC$_{total}$	0.39	0.006	0.38	0.007

Table showing the association between GLP-1 parameters and OGTT-derived indices of insulin secretion and sensitivity. The strength of association of the respective GLP-1 parameter is given as the Pearson's r value (unadjusted model) or β-coefficient (adjusted model) with the corresponding p-value. Only results with significant p-values are presented.

a: Independent variables in the model include age, BMI, ethnicity, smoking status, gestation of OGTT and GLP-1 parameter (GLP-1$_{30min}$, GLP-1 AUC$_{0to30mins}$ or GLP-1 AUC$_{total}$ respectively)

b: As for model 'a' plus GLP-1$_{0min}$

c: As for models 'a' and 'b' but excluding BMI

NS: non-significant; AUC: area under the curve; ISI: insulin sensitivity index; OGIS: oral glucose sensitivity index; OGTT: oral glucose tolerance test